

FIGURE 7.—Weakly acidic compounds in cigarette smoke.

TABLE 16.—Major phenols in cigarette smoke

Phenol	$\mu\text{g/cigarette}$		Remarks ¹
	Nonfilter	Filter	
Phenol	50-130	10-50	1
<i>o</i> -Cresol	20-40	7-20	1
<i>m</i> - + <i>p</i> -Cresol	40-70	15-25	1
2,4-Dimethylphenol	15-25	5-12	1
Catechol	160-500	60-200	2
3-Methylcatechol	15-25	10-20	2
4-Methylcatechol	15-25	10-20	2
Hydroquinone	50-120	N.D. ²	-
Resorcinol	15-20	N.D.	-
Eugenol	3-10	N.D.	-
Isoeugenol	8-20	N.D.	-
Scopoletin	140-280	N.D.	-
Chlorogenic Acid	N.D.	N.D.	-
Rutin	N.D.	N.D.	-
β -Naphthol	0.5-2	N.D.	-

¹Remarks: 1 = Tumor promoting agent on mouse skin
 2 = Cocarcinogen on mouse skin;
 - = Inactive or not tested.

²N.D. = Quantitative data not determined.

SOURCE: Keith, C.H. (52), Morie, G.P. (61).

vapor pressure, no selective reduction by filter tips was observed for catechols (Table 16).

Cyclopentanediones found as constituents of the weakly acidic portion of tobacco smoke are considered important flavor compounds in tobacco smoke. Their concentrations are highest in the smoke of Oriental tobaccos, less in Burley and the least in flue-cured varieties (9:2:1) (26). It appears that these compounds are not toxic.

Carboxylic Acids

A considerable number of carboxylic acids are present in tobacco and tobacco smoke. More than 50 of these have been identified thus far in smoke, accounting for 4 to 7 percent of the particulate matter. The composition of the fraction of volatile carboxylic acids (C_1 to C_6) is a determining factor in the flavor of tobacco varieties. Oriental tobaccos, for example, have a high proportion of β -methylvaleric acid and also contain hydroxyderivatives of valeric- and β -methylvaleric acid. Flue-cured tobaccos are often high in acetic acid, whereas benzoic acid predominates in Burley tobaccos. The non-volatile fatty acids in tobacco range from C_8 - C_{24} with highest concentrations of palmitic acid (C_{16}), C_{18} -acids, stearic, oleic, linoleic and linolenic acids. These range from 0.01 to 0.7 percent in dry tobacco leaf and from 1 to 3 percent in the tar. The highest fatty acid concentrations are found for Turkish tobacco and its smoke.

TABLE 17.—Free fatty acids in cigarette smoke

Acid	$\mu\text{g/l g Tobacco smoked}^1$				
	Turkish 1	Bright	Maryland	Burley	Blend
Palmitic	284	197	107	55	152
Stearic	90	74	43	33	75
Oleic	108	39	32	21	58
Linoleic	146	113	52	50	96
Linolenic	329	310	66	52	240
Total (mg)	0.96	0.73	0.30	0.21	0.62
Wet TPM (mg)	37.2	37.6	26.4	20.1	32.3
5 fatty acids					
% of TPM (wet)	2.6	1.95	1.14	1.05	1.9

¹Moisture content of the tobaccos varied between 11.5 and 12.0%.

SOURCE: Hoffman, D. (40a).

Transfer rates of unchanged fatty acids from tobacco into mainstream smoke can be up to 20 percent, especially for the saturated fatty acids of C₁₆-C₁₈ chain length. Lower transfer rates are observed for the C₁₈ unsaturated fatty acids—oleic, linoleic, and linolenic acid. Comparative concentrations of the major fatty acids in the smoke of various cigarettes are presented in Table 17.

Although high concentrations of fatty acids play a role as tumor promoters in model studies with BaP it appears that these fatty acids are of lesser importance in tobacco carcinogenesis. About two dozen hydroxy- γ -lactones of C₄ to C₆-acids have been identified in tobacco smoke. They probably arise from tobacco leaf carbohydrates by thermal degradation (81). γ -Lactones have not been fully examined for their biological significance in tobacco carcinogenesis. However, several of these compounds are known alkylating agents and as such induce sarcomas in rats (54).

Metallic Constituents

Minerals and other inorganic compounds in the tobacco plant derive from soil, fertilizers, or agricultural sprays. The most prominent metal ions in tobacco are Ca + +, Mg + +, K +, and Na +. During combustion, the bulk of metallic constituents remain in the ashes, but some compounds are vaporized or transferred into the smoke stream. With the growing sophistication of analytical techniques, the list of trace amounts of metals is increasing. Presently, 76 metals, including Bi, Si, As, Se, and Te, excluding the post-uranium metals, have been detected in cigarettes. Of these, 30 have been identified in the smoke (Table 18) (63).

TABLE 18.—Metals in cigarette smoke particulate

Metal	($\mu\text{g}/\text{cig}$)	Metals for which good quantitative data are not available
K	70	
Na	1.3	
Zn	0.36	
Pb	0.24	Si
Al	0.22	Ca
Cu	0.19	Ti
Cd	0.12 ¹	Sr
Ni	0.080 ¹	Tl
Mg	0.070	Po ²
Sb	0.052	
Fe	0.042	
As	0.012 ¹	
Te	0.006	
Bi	0.004	
Hg	0.004	
Mn	0.003	
La	0.0018	
Sc	0.0014	
Cr	0.0014	
Ag	0.0012	
Se	0.001	
Co	0.0002	
Cs	0.0002	
Au	0.00002	

¹Cigarettes other than the University of Kentucky Reference cigarette

²Levels expressed in terms of radioactivity

SOURCE: Norman, V. (69).

With respect to tobacco carcinogenesis, special interest has focused on As and Ni. The continued trend toward replacement of arsenical sprays with other pesticides has been reflected in progressively lower arsenic contents of leaf and smoke. Between 1940 and 1950, arsenic values in the dry leaf of up to 50 to 60 ppm were reported for U.S. tobaccos (31). The last published data for U.S. tobaccos range between 0.5 and 0.9 ppm (28). Between 7 and 18 percent of the total arsenic in tobacco reappears in the mainstream smoke of cigarettes. Studies with ⁷⁴As-labelled cigarettes have shown that, depending on the individual's smoking patterns, 2.2 to 8.6 percent of the arsenic in cigarette tobacco is transferred into the respiratory tract. About 50 percent of the inhaled arsenic is eliminated within 10 days, primarily in urine; the remainder is either deposited in body tissues or is exhaled or otherwise eliminated (41).

All forms of nickel (metal, oxide, sulfide, salts, and carbonyl) tested in the experimental animal were found to be carcinogenic. In nickel factories, primarily in those converting nickel sulfide to nickel oxide,

workers have a high risk for cancer of the nasal cavity and cancer of the lung. In cigarette tobacco, 2.0 to 6.2 μg Ni per cigarette were reported; other tobacco products contained between 0.5 and 8.5 μg per gram. In South Africa, nickel values of 52 and 88 μg per gram of Swazi snuff were reported as a possible contributing factor in the high incidence rate of cancer of the nose and in accessory sinuses in male Bantus (5). During smoking, 10 to 20 percent of the nickel in the tobacco is transferred into the mainstream smoke (62). In one study, tentative evidence indicated that most of the nickel transferred into the mainstream smoke ($\cong 10$) is present in the gas phase ($\cong 8$ percent) (90). This and a model study suggest that nickel is present in the gas phase of tobacco smoke as nickel carbonyl. $\text{Ni}(\text{CO})_4$ is highly carcinogenic in the respiratory tract of rats. It induces epidermoid carcinomas and adenocarcinomas of the lung (89).

Several forms of cadmium are carcinogenic in the experimental animal. Two studies suggest that occupational exposure to cadmium oxide may increase the risk of prostate cancer (45). In mainstream smoke, concentrations are 9–70 ng Cd per cigarette (45). It has been suggested that a heavy smoker retains about 1.5 μg of Cd per day and that he may accumulate up to 0.5 mg through inhalation.

Radioactive Compounds

Two types of radioactive compounds have been reported in tobacco and tobacco smoke. These are the α -particle emitting elements of the disintegrating radium and thorium series and the β -emitters. In the latter group, potassium-40 is the most abundant in tobacco products (100). A sample of 100 U.S. and Canadian cigarettes was found to contain 2,120 and 2,295 pCi of ^{40}K -derived β -activity, respectively. The β -activity from ^{40}K in the mainstream smoke of 100 cigarettes was 15.9 and 9.4 pCi, corresponding to a transfer rate of 0.75 percent and .41 percent, respectively. ^{40}K is a soft emitter with E_{max} of 1.3 meV.

The presence of radioelements ^{226}Ra , ^{210}Pb , and ^{210}Po in tobacco products (e.g., from fallout, natural background) have been of special interest and concern (69). The general range of ^{210}Po in 1 g of U.S. tobacco leaf varies from 0.15 to 0.45 pCi. In the smoke of one U.S. cigarette, ^{210}Po values of between 0.03 and 0.07 pCi were reported. The average ^{210}Po content was $\cong 0.036$ pCi per cigarette or $\cong 2.6$ pCi of ^{210}Po per 1 g smoke condensate with a ^{210}Pb : ^{210}Po ratio of 0.66 ± 0.23 (42). $^{210}_{82}\text{Pb}$ has a half-lifetime of 22 years and decays by emission of two β -particles to $^{210}_{84}\text{Po}$; the latter decays by α -emission with a half-lifetime of 138.4 days. Preliminary studies indicate that most of the ^{210}Pb is concentrated in the nonvolatile and insoluble portion of the particulate matter of cigarette smoke (58).

Analysis of human tissues demonstrated that the lung, blood, and liver of smokers contain a higher concentration of ^{210}Po than do those of nonsmokers. It has been calculated that a smoker's intake of ^{210}Po is

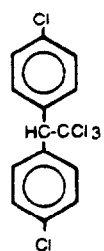
reflected within several days by the observed excess burden of 3–10 pCi of ^{210}Pb and ^{210}Po in the lungs. Based on the measured concentration of ^{210}Po in epithelial samples, Little and Radford estimated a maximum radiation dose of $\cong 200$ rem per 25 years to the lower lobe bifurcations of the lung (56); however, others have estimated a far lower effective radiation dose (14, 70).

After multiple intratracheal installations of ^{210}Po in Syrian golden hamsters, a dose-dependent increase was observed in epidermoid carcinoma and adenocarcinoma in the peripheral lung fields (55). Simultaneous and multiple intratracheal instillation of benzo(a)pyrene (total dose 4.5 mg) and ^{210}Po (total dose 50,000 pCi) on the same carrier induced twice the number of tumors expected from the additive effect of either carcinogen alone (59).

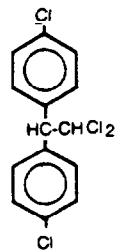
Agricultural Chemicals

As in the case of arsenical pesticides, a significant reduction in the use of chlorinated hydrocarbon insecticides on tobacco has occurred during the last decade. This is reflected in the reduction of such insecticide residues as DDD, DDT, endrin, and endosulfan on tobacco (Figure 8). Whereas in 1968 70.2 percent of all U.S. flue-cured auction-marketed tobaccos contained more than 10 ppm of DDT, in 1972 there was no tobacco of the same type containing levels above 10 ppm of DDT. In the latter year, 73.1 percent of the tobaccos marketed contained only 0.1 to 0.49 ppm of DDT (17). DDD values declined from levels of ≥ 10 ppm in 97.6 percent of the 1968 crop to levels no higher than 0.1 to 0.49 percent in 63.9 percent of the tobaccos marketed in 1972. Again, there was no tobacco with levels of DDD above 10 ppm in 1972. Similar reductions of insecticide residues on tobacco were reported for endrin, dieldrin, and endosulfan (17, 30). A further gradual decrease of these pesticides in tobacco is expected. During smoking, 11 to 18 percent of DDT and DDD are transferred without change of structure from tobacco into the mainstream smoke of cigarettes. DDE, DDM, and 4,4'-dichlorostilbene (Figure 8), an immediate decomposition product of DDT and DDM resulting from elimination of HCL and molecular rearrangement, are also detected in mainstream smoke (39). One study showed that levels of chlorinated hydrocarbon insecticides in adipose tissues of smokers were not elevated above those in nonsmokers (18). Other pesticide residues found on some U.S. tobaccos are parathion (up to 0.03 ppm), carbaryl (up to 1.5 ppm), endosulfan (up to 2.9 ppm), and toxaphene (0.7 to 3.4 ppm) (30).

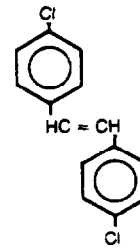
Some of the chlorinated hydrocarbon insecticides and the isomeric impurities present in the technical preparations, e.g., o,p'-DDD, are possible or known carcinogens in experimental animals. One of the co-carcinogens is 4,4'-dichlorostilbene, formed by pyrolysis from DDT and DDD (40). As discussed earlier, the carbaryl residue on tobacco may give rise to a carcinogenic nitrosamine. Similarly, maleic hydrazide and



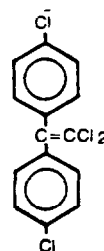
DDT



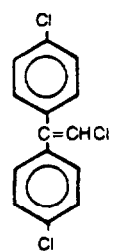
DDD



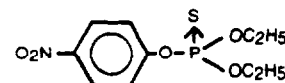
4,4'-Dichlorostilbene



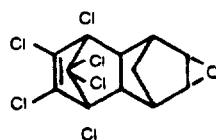
DDE



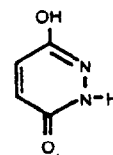
DDM



Parathion



Endrin



Maleic Hydrazide (MH)

FIGURE 8.—Residues of agricultural chemicals in tobacco and cigarette smoke.

its soluble salts have been mentioned. Present evidence is not uniformly clear as to whether pure MH is mutagenic or carcinogenic, though the weight of the evidence suggests it is mutagenic. (22, 32).

Tobacco Additives

Tobacco products are refined by the addition of additives, humectants, tobacco casings, and flavor-enhancing compounds. The most widely used humectants are propanediol, glycerol, diethylene glycol, triethylene glycol, and D-sorbitol (100). Humectants amount to 2 to 4 percent of the original tobacco weight for cigarettes. Analyses of 18 U.S. cigarette brands showed ranges of 0.46 to 2.24 percent of propylene glycol and 1.7 to 3.15 percent of glycerol in the tobaccos (15). Smoke analyses demonstrated that in filter cigarettes 9.9 percent and in nonfilter cigarettes 12.6 percent of the propylene glycol in tobacco reappear unchanged in the mainstream smoke. The glycerol transfer rate into the mainstream smoke of filter and nonfilter cigarettes was 12 and 14 percent, respectively. The smoke of humectant-treated cigarettes had increased amounts of acetaldehyde and acetone (53). Transfer of humectants into the mainstream smoke is probably significantly greater in pipe smoking than in cigarette smoking because of the former's higher puff frequency (60).

The use of humectants in tobacco products has raised concern as to their effects on smoke toxicity. Formation of volatile aldehydes and ketones, including acrolein, from combustion of such humectants would add to the ciliotoxicity of tobacco smoke. The glycols, especially diethylene glycol, are suspected to influence the smoker's risk for bladder cancer (44).

Pipe tobaccos may contain up to 30 percent of casing agents. These are primarily sugars, starches, humectants, and plant extracted isoprenoids. These casing agents influence the flavor of the tobacco smoke, as well as the burning rate of the tobacco, and thus affect smoke toxicity. When cigarette tobacco contained 5 percent or higher levels of sugar additives, the resulting smoke was higher in furfural, nicotine, and tar content than the smoke from an identical cigarette without the sugar casing (86).

The flavor of cigarette smoke is also affected by the curing, aging, and blend of tobaccos used. Considerations such as acreage yield and tobacco prices during the last decade have resulted in changes of leaf aroma affecting the tobacco blends and thus the smoke flavor. More importantly, however, the trend toward low-tar, low-nicotine cigarettes and toward a reduction of undesirable volatile smoke compounds has brought about major changes in the smoke flavor of cigarettes. The use of rolled stems and reconstituted tobacco sheet admixed with leaf lamina and the use of effective filter tips are major factors inducing changes in smoke flavor. All of these developments have led to increased use of flavor additives, especially for low-tar, low-nicotine

TABLE 19.—Harmful constituents of cigarette smoke particulate matter

I. Compounds judged most likely to contribute to the health hazards of smoking ¹ :		
Nicotine	50–2,500 µg/cig	"Tar" ² 500–35,000 µg/cig
II. Compounds judged as probable contributors to the health hazards of smoking:		
Cresols (all 3 isomers)		Phenol 9–202 µg/cig
	68–97 µg/cig	
III. Compounds judged as suspected contributors to the health hazards of smoking:		
DDT	0–0.77 µg/cig	Endrin 0–0.06 µg/cig
Hydroquinone	83 µg/cig	Nickel compounds 0–0.58 µg/cig
Pyridine	25–218 µg/cig	

¹Values from May 1978 FTC list

²"Tar" contains the polynuclear aromatic hydrocarbons which are "generally accepted as being responsible for a substantial portion of the carcinogenic activity of the total "tar". "Tar" also contains β-naphthylamine, a known human bladder carcinogen for which there is no known safe level of human exposure.

SOURCE: U.S. Public Health Service (93).

cigarettes. In fact, these new cigarettes require flavor corrections by additives in order to be acceptable to the consumer. Tobacco extracts as well as nontobacco flavors, such as licorice, coca, fruit, spices, and floral compositions, are used. More recently, suggestions for synthetic flavor additives for cigarette tobaccos are increasing in the patent literature. At present, the selection of tobacco flavor additives from the GRAS (Generally Regarded As Safe) List or from natural extracts and the screening of their smoke decomposition products for toxicity or other biological activity are not required by law and are done voluntarily by manufacturers.

Toxic and Carcinogenic Agents—A Summary

The report of an expert panel on the "harmful constituents of cigarette smoke" classified the harmful and possibly harmful smoke compounds into the following categories: (1) contributors, (2) probable contributors, and (3) suspected contributors to the health hazard of smoking (93).

The constituents of the particulate matter are listed according to this classification in Table 19. Since 1970, when the harmful smoke constituents were so defined, much progress has been made toward the identification of toxic and especially of tumorigenic agents in cigarette smoke. The identified tumorigenic agents and their quantities in cigarette smoke are listed in Table 20. The majority of co-carcinogenic agents in cigarette smoke remain to be identified.

The increased risk for cigarette smokers of cancer of the esophagus, kidney, and urinary bladder suggests the possibility that cigarette

TABLE 20.—Known tumorigenic agents in cigarette smoke particulates

Compound	µg/cig	Compound	µg/cig
<i>I. Tumor Initiators</i>		<i>II. Co-carcinogens</i>	
Benzo(a)pyrene	0.01–0.05	Pyrene	0.05–0.2
Other PAH ¹	0.3–0.4	Other PAH ²	0.5–1.0
Dibenz(a,j)acridine	0.003–0.01	1-Methylindoles	0.8–
Other Aza Arenes	0.01–0.02	9-Methylcarbazoles	0.14–
Urethane	0.035	4, 4-Dichlorostilbene	0.5–1.5
		Catechol	200–500
		Alkylcatechols	10–30
<i>III Organ Specific Carcinogens</i>			
N'-Nitrosornicotine	0.14–3.70		
4-(N-Methyl-N-nitroso-amino)-1-(3-pyridyl)-1-butanone	0.11–0.42		
N'-Nitrosoanatabine	+ ³		
Polonium-210	0.03–0.07pCi		
Nickel Compound	0–5.8		
Cadmium compounds	0.01–0.07		
β-Naphthylamine	0.001–0.022		
4-Aminobiphenyl	0.001–0.002		
O-Toluidine	0.16		

¹For details see Table 15

²For details see Table 15

³Concentrations unknown

SOURCE: U.S. Public Health Service (33).

smoke contains unidentified organ-specific carcinogens besides the known trace amounts of carcinogenic aromatic amines and N-nitrosamines.

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Physiological Responses to Cigarette Smoke

Previous editions of this report have examined acute and chronic effects of cigarette smoke. Starting with epidemiological evidence and buttressed by clinical and pathological findings, the role of cigarette smoke has been implicated in numerous disease processes in humans.

Since smoke is such a complex mixture of elements, experimental work in humans must be augmented by animal studies in order to define the specific role of particular smoke components. Inhalation studies (33) must be designed to closely mimic smoke exposure in the human population and provide data relating to: (1) understanding the physiological or biochemical mechanism of action of whole cigarette smoke or individual smoke components, (2) understanding of pathogenesis and early identification of endpoints which are predictive in nature, and (3) screening potentially less hazardous cigarette models to differentiate their relative influence on physiological or pathological endpoints.

Bioassays must be designed with appropriate exposure modes, since cigarette smoke-related diseases in man are usually chronic and involve a history of prolonged interaction between smoke components and target tissue.

Animal Smoke Inhalation Exposure Methodology

Smoke Generation

Exposure systems for tobacco smoke can be classified as active or passive, depending upon the system used for generating cigarette smoke.

Active exposure systems require the animal to generate the smoke by drawing air through a lighted cigarette to simulate what happens to the human smoker. McGill, et al. (30) used a water-reward system to train baboons to puff on lighted cigarettes and to inhale cigarette smoke. Once the animals were trained to take puffs of a specific duration, it was possible to control the animal's smoking behavior by manipulating the water reward per puff. The effectiveness of this system was shown by the fact that the animals remained in good health throughout the period of training and were able to achieve blood carboxyhemoglobin levels similar to those of human smokers.

However, since most experimental animals will not cooperate as well as baboons, passive devices in which smoke is generated by a machine are commonly used. Passive exposure systems can then be further classified as continuous or intermittent. A continuous system is one which smokes a series of cigarettes at one time by using one or two rotating discs or turrets to position the cigarettes at a smoking port where the puff is usually drawn by a vacuum pump. By designing the system so that a cigarette on one turret is being smoked while a

cigarette on the other turret is being rotated into position, it is possible to generate a nearly continuous stream of smoke (24).

In the intermittent system, smoke is generated either by applying positive pressure to a chamber containing a cigarette and forcing smoke out through the cigarette (36) or by a cam-activated plunger which draws a puff of smoke and injects it into a holding tube (4) where it is allowed to stand. The smoke generated by the piston is a closer approximation of the human smoke generation process than earlier mechanical smokers. It can be more accurately controlled as to puff volume, duration, and frequency and thus is the currently preferred system.

Methods of Inhalant Delivery

A great number of different exposure systems are available for tobacco smoke inhalation experimentation. Since the goal of much of the inhalation research currently being done is intended to simulate human experience, some degree of compromise is usually involved in selecting an inhalation system. The basic systems for delivering tobacco smoke inhalants include:

(1) complete chamber exposure—the entire animal is exposed to the inhalant (6, 36).

(2) partial chamber exposure—only the nose of the animal is exposed to the inhalant (29).

(3) face mask or mouth piece exposure—the inhaled smoke is delivered to the nose or mouth through a mask or mouthpiece, with a means of allowing expired smoke and air to be exhausted (8, 35).

(4) tracheal exposure—the inhalant is delivered directly into the trachea via a cannula inserted into a permanent tracheotomy (12).

The decision to use a particular exposure system is made after considering factors such as selection of a suitable animal model; the ability to control exposure levels, including delivery of smoke as a bolus in a fresh air stream; system wash-in and wash-out times; the ability to sample inhalant and/or test gases from the system inlet or outlet; and the ability of the exposure system to deliver smoke to the experimental animal while offering the least alteration of normal respiratory function.

Dosimetry

Administration of experimental inhalants via the pulmonary route requires a description of the concentration, duration, and pattern of inhalant exposure. Unfortunately, there is no simple relationship among these variables that will determine the dose delivered to a specific site of interest in the experimental animal. Prime attention must be given to the definition of real-life human exposure conditions so that appropriate parameters can be incorporated into the experi-

ment, although as noted by Nettesheim, et al. (33), the investigator determines the smoke exposure conditions but the animal determines smoke uptake or dose.

Periodic measurements to determine the amounts of cigarette smoke components received by experimental animals can be just as complex and equally as important as the endpoints used in the characterization and evaluation of the effects of tobacco smoke exposure.

Among the indicators which have been used for monitoring smoke uptake are blood levels of nicotine (20), urinary nicotine and cotinine (11), and tracers such as decachlorobiphenyl (6, 7) and ¹⁴C-dotriacontane (15). Each of these indicators has problems associated with it, such as the need for lengthy extractions for nicotine and cotinine and the requirements for homogenation of tissue samples prior to determining decachlorobiphenyl content.

Blood carboxyhemoglobin (COHb) levels have often been given to indicate that animals have inhaled the smoke, since carbon monoxide absorption occurs primarily in the lungs. In a study of total particulate matter (TPM) deposition in the lungs of small mammals, Binns, et al. (6) also examined COHb levels to determine the correlation between these tests. They found that TPM could only be predicted from COHb levels within fairly wide levels in a particular species and showed no clear relationship when comparing different species.

Limiting Factors in Smoke Exposure

The major factor limiting the size of the dose in cigarette smoke inhalation studies is the acute toxicity of carbon monoxide and nicotine (39). In developing exposure regimens, it is important to consider acute toxicity of these two substances as well as the irritant nature of smoke when it is delivered to animals in high concentrations (7).

Excessive carbon monoxide buildup in blood, which can alter the transport of oxygen of the experimental animal, is a common problem in continuous exposure systems. To prevent toxicity of smoke, such systems require excessive dilution or intermittent exposure, which can lead to exposures of animals to smoke of different chemical and physical properties. Although the same situation is true for acute toxicity of nicotine, its half life is much shorter than that of carbon monoxide.

Intermittent systems have also been found to be advantageous in smoke exposure studies. These systems operate on a puff-hold-purge cycle with a holding period which can be adjusted to prevent major chemical and physical changes in the smoke. Rylander (38) has reviewed some of the contradictory results which occurred with varied smoke exposure conditions and has stressed the need to monitor smoke dilution, exposure duration, and selective absorption of volatile water-soluble smoke constituents.

Selected Animal Studies

Pulmonary Studies

Since Cahan and Kirman (12) published a method of delivering smoke to dogs in a controlled manner, the dog has been widely used as an animal model. While their report was primarily a technique paper, the authors noted increases in hematocrits and cardiac hypertrophy along with pulmonary fibrosis and emphysema in the smoking group.

A further description of pulmonary morphologic changes induced by smoking was published by Frasca, et al. (22). Their electronmicroscopic findings included a complete loss or marked reduction in the number of capillaries and a marked thickening of the septa due to increased amounts of collagen in the lung parenchyma. They also found large numbers of macrophages in both the pleura and parenchyma, occurring singly and in clumps. Many of these macrophages contained crystalline-like structures in membrane-bound inclusions.

Male cynomolgus monkeys trained to smoke an average of 12 cigarettes a day for 5 days a week over 6 months showed no changes in their epithelia of large airways but did exhibit aggregation of a large number of macrophages in the alveoli (8). These macrophages were clumped, pigmented with black/brown granules, and had foamy cytoplasm. Pulmonary physiological changes were limited to increases in pulmonary resistance, while tidal volume, respiratory rate, dynamic compliance, and nitrogen washout were normal throughout the test period.

Park, et al. (35) found that pulmonary mechanics and arterial blood gases of dogs which smoked eight cigarettes per day showed no significant differences until after 11 months of smoking, when functional residual capacity fell slightly and respiratory resistance rose. They attributed these changes, in part, to the smaller lung size of the smoking dogs. As in earlier studies, an increased number of alveolar macrophages were harvested from the lungs of smokers. Functional changes in macrophages included an increased initial latex uptake and a decreased bacteriosuppressive activity in smoking dogs.

Cardiovascular Studies

Chronic changes in cardiovascular functions due to tobacco smoke have not been extensively investigated in intact animals. A study by Ahmed, et al. (1) compared hemodynamics and left ventricular microscopic structural changes after beagle dogs smoked seven cigarettes per day or were given an equivalent intramuscular dose of nicotine daily for 22 months. They reported that both experimental groups had smaller left ventricular ejection fractions and lower left ventricular dP/dt values, both of which reflect a deficit in the contractile function of left ventricular muscle. Mean aortic blood pressure was elevated in both groups, indicating an increased peripheral resistance. Since the left

ventricular contractility indices were still lower after acute phlebotomy, it appeared that the left ventricular function was compromised independently of the increased afterload. The only histological change was an increased amount of collagen in the interstitium.

Armitage (2) administered puffs of smoke to anesthetized or spinal cats and demonstrated transient increases in blood pressure. By comparing these pressure changes with those observed when intravenous injections of nicotine were given, he was able to obtain an estimation of the pharmacologic "dose" of nicotine-like substance(s) contained in a puff of smoke. The study demonstrated that the source of the pressor response was in the particulate phase of the smoke although it may not have been nicotine per se, since smoke from low-nicotine cigarettes caused increased blood pressure similar to smoke from a cigarette with a standard nicotine level.

The role of tobacco smoke in altering myocardial oxygen partial pressure (MP_{O_2}) was studied by Rink (37) in a series of experiments in open-chested cats with implanted oxygen electrodes. Intravenous injections of nicotine or intratracheal puffs of smoke resulted in transitory increases of blood pressure and slight increases in MP_{O_2} . It was postulated that the effect of lower oxygen availability due to CO in tobacco smoke was overshadowed by the actions of nicotine in increasing myocardial blood supply.

The preceding studies have all indicated the adaptive nature of the animal or organ system under study. While compensatory mechanisms may serve to minimize the acute or chronic insult of tobacco smoke or its specific components, the underlying assumption has been that the system is "normal" or "healthy" and thus able to respond.

To examine the effect of tobacco smoke on an impaired cardiovascular system, Bellet, et al. (5) produced myocardial infarcts in dogs by ligating the anterior descending branch of the left coronary artery. After allowing four days for recovery, ventricular fibrillation threshold (VFT) was determined in control and smoking dogs with and without infarcts. As expected, VFT was lower in dogs with myocardial infarcts. In both control dogs and in dogs with acute myocardial infarction, inhalation of cigarette smoke decreased VFT for up to 90 minutes after exposure. The authors noted that the effects of myocardial infarction and cigarette smoke on the VFT were additive.

Exercise Tolerance

To investigate smoke-related impairments in physical exertions, animals have been subjected to exercise programs involving swimming or running on a treadmill before and after smoking. Hrubes and Battig (26) trained rats to swim to the point of exhaustion. As the animals became adapted to the program, endurance times rose from 5 to 7 or 8 minutes, but after acute smoke exposure, the endurance times fell to 5 minutes.

Reece and Ball (36) examined electrocardiographic, blood enzyme, and hematological data on dogs which ran on a treadmill for 10 minutes a day for a year. In the smoking group, electrocardiographic change indicated cardiac enlargement, suggestive of left ventricular hypertrophy. Of the enzymes studied, postexercise lactate concentrations rose after smoke exposure began, reflecting a deficiency in oxygen transfer, transport, or utilization, all of which occur with carbon monoxide exposure. Other enzymes altered during smoke exposure included glutamic oxaloacetic transaminase and creatine phosphokinase. While there was no histopathological basis for these changes, the authors noted the potential for the combination of hypoxia and nicotine to inhibit the production of certain enzymes.

Toxicity of Specific Smoke Components

Since the list of harmful constituents in cigarette smoke was published in 1972 in the report *The Health Consequences of Smoking*, there has not been a notable increase in knowledge regarding the pathophysiological role of many specific smoke components.

Rylander (38) reviewed experimental work dealing with aerosol and volatile components of smoke and listed three requirements for determining relative toxicity: (1) realistic dilution of the smoke as drawn from cigarettes, (2) selective absorption of volatile, water-soluble compounds from the smoke, and (3) realistic exposure duration.

These same criteria should apply to examination of specific components of tobacco smoke. Many studies such as those which determined LD₅₀ levels or reported results of continuous exposures were considered not to represent smoke-related results.

Nicotine

In an early study to determine how nicotine in cigarette smoke could cause an increase in heart rate, Burn and Rand (10) administered nicotine to isolated rabbit atria. By comparing normal and reserpine-treated atria, they found that nicotine caused increases in rate and amplitude of contraction by releasing epinephrine and norepinephrine from stores in the heart. Interest in the role of nicotine in cardiovascular diseases processes has continued from that time, aided in part by the availability and ease of administration of pure nicotine solutions.

Ilebekk and Lekven (27) used a continuous infusion of nicotine to examine the mechanical efficiency of the left ventricle during the administration of approximately 2.1 mg of nicotine over a 5-minute period. They found that nicotine increased cardiac contractility and elevated left-ventricular-systolic and end-diastolic pressures. Thus, even though peripheral vasoconstriction occurred, stroke volume was increased by nicotine during these short-term studies.

By comparing chronic smoke exposure and daily intramuscular injections of nicotine, Ahmed, et al. (1) were able to demonstrate that left ventricular performance did deteriorate over the course of 22 months. Ahmed reported that aortic blood pressure rose in both test groups, so that nicotine appeared to be involved in the increased peripheral resistance. Since both the smoking and nicotine groups exhibited similar interstitial fibrosis in the middle layers of myocardial tissue, nicotine appears to have a cardiotoxic effect which has previously been ascribed to carbon monoxide.

The association between nicotine and hypertension is not as clearcut as the two preceding reports may suggest. Fisher, et al. (21) investigated the role of nicotine in atherosclerosis and experimental hypertension in rabbits and found nicotine had no effect on either disease process over a 90-day period. While others had reported no link between nicotine and atherosclerosis, the authors noted that the dose of nicotine may not have been optimal to allow comparison with previous work in the area of hypertension.

A report by Hansson and Schmitterlow (25) examined the distribution of nicotine in various tissues and noted that the metabolism of nicotine in isolated tissue slices was oxygen-dependent. In a study of nicotine conversion rates in intact rats, Miller, et al. found that, while plasma nicotine clearance rates were independent of peak plasma levels (31), dose-dependent differences of nicotine distribution in tissues resulting from changes in regional perfusion may have effected total plasma clearance of nicotine. It thus appears likely that selective oxygen availability as well as plasma nicotine levels may influence nicotine catabolism in experimental animals.

Carbon Monoxide

When pregnant rats were maintained in a CO atmosphere that produced carboxyhemoglobin levels averaging 15 percent saturation, their offspring exhibited reduced birth weights, decreased weight gains, and lower brain protein levels than air-breathing controls (19). While this study might be criticized for using continuous rather than intermittent exposures, the data do suggest a highly sensitive indicator of CO toxicity.

Additional study of carbon monoxide toxicity also pointed out another case of relative susceptibility, again using the rat bioassay. When comparing tracheal pressure, blood pressure, and heart rate responses in guinea pigs and rats exposed to 2.84 percent carbon monoxide, Mordelet-Dambrine, et al. (32) noted that rats appeared to be more sensitive, since they had lower survival times. These differences may be due to differences in CO sensitivities, or they may be due to anesthetic variables that are hard to quantitate across species.

To avoid anesthetic problems, Cramlet, et al. (13) used conscious dogs that were chronically instrumented to provide continuous cardiovascular data with cannulae for blood sampling from left and right atria while the dogs inhaled carbon monoxide. Measurements were made when COHb reached 10, 20, and 30 percent saturation. The only significant cardiac changes were heart rate increases at 20 and 30 percent saturation; arterial oxygen saturation was reduced at all levels. The authors concluded that cardiac compensation was adequate to prevent tissue hypoxia up to 30 percent COHb in healthy dogs.

In an effort to study the effects of carbon monoxide in dogs with impaired hearts, DeBias, et al. (18) produced myocardial infarcts by injecting latex spheres into the left coronary artery. Control and infarcted dogs were exposed to carbon monoxide continuously for 14 weeks with serial electrocardiograms and hematologic evaluation. Although COHb averaged 14 percent in exposed animals, the animals remained in good health throughout the study.

Repeating the same protocol in cynomolgus monkeys, DeBias, et al. (17) found hematocrit, RBC, and hemoglobin levels altered by 3 weeks of exposure to 100 ppm CO, with recognizable electrocardiographic changes. The authors concluded that the sensitivity to CO was species-related as well as dose-related.

Carrying these results one step further, the DeBias group (16) examined the effect of carbon monoxide on ventricular threshold in cynomolgus monkeys. Animals with and without myocardial infarcts produced by latex bead injections into the coronary artery were exposed to 100 ppm CO for 6 hours. This CO level produced COHb values of 9.3 percent compared to 1.1 percent in air-breathing animals. It was noted that infarcted and CO-breathing animals both had lower ventricular fibrillation thresholds, and that the effects were additive.

The lack of chronic studies on CO effects in animals and humans suggests that such studies be undertaken to fill this void in our knowledge, especially as it relates to smoking and related diseases.

Nitric Oxide

While nitric oxide is found in cigarette smoke in concentrations of zero to 600 μg /cigarette (39), blood levels for humans, monkeys, and rats have only recently been reported (23). Their data indicate that a consistently low level of NO was maintained in the blood of both smokers and nonsmokers. The lack of a significant difference between smokers and nonsmokers suggests that a mechanism exists in mammals to rapidly detoxify NO, and that exogenous NO appears to have little effect on its steady state in blood.

Examining the role of NO at the cellular level, Arnold, et al. (3) exposed tubes containing rat and bovine tissue to the gas phase of cigarette smoke, nitric oxide, and room air and determined changes in guanylate cyclase activity. This enzyme is involved in the formation of